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# Diagnostic Value of Some Biochemical Bone Markers for the Detection of Bone Metastases in Prostate Cancer

*Lambertus W. J. J. M. Westerhuis*[1] and *Karl P. J. Delaere*[2]

[1] Department of Clinical Chemistry, Ziekenhuis De Wever & Gregorius, Heerlen, The Netherlands
[2] Department of Urology, Ziekenhuis De Wever & Gregorius, Heerlen, The Netherlands

**Summary:** Bone metastases in cancer of the prostate are diagnosed routinely by isotope bone scintigraphy and the measurement of alkaline phosphatase in serum and the calcium excretion in urine. The specificity of these examinations is in general not satisfactory. We therefore investigated the diagnostic value of five new markers of bone formation and bone resorption for the detection of the metastatic process. In a group of 43 patients with carcinoma of the prostate the carboxyterminal propeptide, the carboxyterminal cross-linked telopeptide, the aminoterminal cross-linked telopeptide, and the deoxypyridinoline cross-links of type 1 collagen were measured as well as the specific bone alkaline phosphatase isoenzyme. A group of 34 patients with benign prostatic hyperplasia served as a control. A receiver-operating characteristic analysis was performed. It appeared that the sensitivity of carboxyterminal cross-linked telopeptide of type I collagen was the greatest (89%), while the best specificity was obtained for the deoxypyridinoline cross-links assay (92%). The diagnostic values of the new markers were generally comparable with those of alkaline phosphatase although carboxyterminal cross-linked telopeptide of type I collagen yielded better results, but those with carboxyterminal propeptide of type I procollagen were less satisfactory. Calcium excretion in urine had no added value at all.

## Introduction

Metastatic bone disease is one of the most dominant extensions in adult patients with malignancy. In particular, patients with carcinoma of the prostate are at risk for metastatic bone involvement. The median survival is about 2.5 years from the time of diagnosis of bone metastases and 80% of patients who die from prostate cancer have bone metastases (1). Until now the diagnosis of bone metastases has mainly relied on isotope bone scintigraphy. Although bone scintigraphy has a high sensitivity, the specificity appears to be low and it is difficult to quantitate routinely the uptake of isotope (2).

Collagen type I is the most abundant protein in bone, so it is not surprising that the use of biochemical collagen markers has been investigated in metastatic bone disease. Several authors have recently reported not only increased production of collagen formation markers, but also of collagen resorption markers in patients with metastatic bone disease (3–7). In general it appears from these studies that the specificity of the collagen markers is rather poor, reducing their diagnostic value for detection of the metastatic process in bone. However, the patient groups in these studies were very heterogeneous with respect to their primary malignant disease and only very few patients with carcinoma of the prostate were included. This might be an explanation for the low overall specificity of the collagen markers studied.

In a well-defined group of patients with metastatic prostate cancer it was recently demonstrated that the levels of carboxyterminal cross-linked telopeptide of type I collagen and of carboxyterminal propeptide of type I collagen were considerably higher than the normal reference values and that carboxyterminal cross-linked telopeptide of type I collagen did yield some information about the prognosis of the disease (8). This study did not contain any patients who had prostate cancer without bone metastases and there was no control group, so no data were presented on the specificity or sensitivity of the markers studied. Another recent study (9) showed elevations of collagen markers in patients with prostatic bone metastases compared with patients without them. However, a considerable overlap between the results of the two groups caused a low sensitivity.

In order to establish the diagnostic value of some biochemical bone markers for the detection of bone metastases in prostate cancer we performed a receiver-operating characteristic analysis for some markers of bone formation and of bone resorption in two groups of patients with prostate cancer (with or without bone metastases). A group of patients with benign prostatic hyperplasia served as a control group. In this study, the bone formation markers alkaline phosphastase, specific bone alkaline phosphatase and carboxyterminal propeptide of type I procollagen were measured together with the bone

resorption markers carboxyterminal cross-linked telopeptide of type I collagen, the urinary calcium excretion, deoxypyridinoline cross-links and the amino terminal telopeptide of type I collagen.

## Patients and Methods

In total, 77 patients gave informed consent for this study. Thirty-four patients had a clinically diagnosed benign prostatic hyperplasia and 43 patients had cancer of the prostate, confirmed histologically after a biopsy or radical prostatectomy. All patients with cancer underwent 99-m-technetium bone scintigraphy and were, according to the results of this examination, divided into two groups PC− and PC+, without and with bone metastases. Blood samples were taken in the morning between 8.30 a. m. and 9.30 a. m. Blood was collected in plain plastic tubes, allowed to clot and serum was separated from erythrocytes by centrifugation at 4 °C, for 10 min at 1500 *g*. The samples were divided for the different determinations and frozen at −70 °C prior to assay. Urine samples for the determination of deoxypyridinoline cross-links, aminoterminal telopeptide of type I collagen and calcium excretions were obtained from an overnight urine portion including the early morning portion. After division into aliquots, the urine samples were also frozen at −70 °C before assay. Urinary concentrations were expressed relative to the urinary creatinine levels.

### Alkaline phosphatase

Activity of total alkaline phosphatase in serum was measured at 37 °C, following the recommendations of the IFCC, using a Cobas Integra Analyzer (Roche, Switzerland).

### Bone alkaline phosphatase

Bone alkaline phosphatase in serum was determined by means of an enzyme immuno-assay according to the manufacturer's instructions (Alkphase-B, Metra Biosystems, Palo Alto, USA).

### Carboxyterminal propeptide of type I procollagen/ carboxyterminal cross-linked telopeptide of type I collagen

The concentrations of carboxyterminal propeptide of type I procollagen and carboxyterminal cross-linked telopeptide of type I collagen in serum were assessed by commercially available radio-immuno-assays according to the manufacturer's instructions (Orion Diagnostica, Finland).

### Deoxypyridinoline cross-links

Urinary concentration of free deoxypyridinoline was determined by a competitive enzyme immuno-assay (Pyrilinks-D, Metra Biosystems, Palo Alto, USA) according to the manufacturer's instructions.

### Aminoterminal telopeptide of type I collagen

Urinary concentration of the aminoterminal telopeptide was determined by ELISA (Osteomark) according to the manufacturer's instructions (Ostex International, Seattle, WA, USA).

### Calcium and creatinine in urine

Urinary calcium concentration was determined by means of atomic absorption spectrophotometry (Perkin Elmer 2380) and urinary creatinine was determined by the alkaline picrate method using a Cobas Integra Analyzer (Roche, Switzerland).

### Prostate-specific antigen

The level of prostate-specific antigen in serum was determined with a fluoro-immunometric assay on an AutoDelfia automatic immuno-assay system according to the manufacturer's instructions (Wallac OY, Turku, Finland).

### Statistical analyses

All analytes measured are presented as medians and ranges. The significance of differences between groups of patients was assessed using the *Mann-Whitney* U-test. The probability of falsely declaring one or more differences to be significant caused by multiple comparisons between groups was eliminated by applying the principle of *Bonferroni*'s inequality (10). This means that the significance level was adjusted for the number (n = 3) of comparisons between the patient groups. The different quantities were correlated with each other with the *Spearman* rank correlation test. The sensitivity and specificity of the analytes for predicting metastatic bone involvement (according to scintigraphic criteria) were calculated for several arbitrarily chosen cutoff levels. Receiver operating-characteristic curves were constructed by plotting the sensitivity against 100 minus the specificity. The resulting areas under the receiver operating-characteristic curves were used as a measure for comparing the diagnostic values of the different analytes.

## Results

Table 1 shows some characteristics of the patient groups. The group of patients with benign prostate hyperplasia appeared to have normal prostate-specific antigen values (< 10 µg/l). On the other hand, practically all patients with carcinoma of the prostate appeared to have elevated prostate-specific antigen values at the time of diagnosis of the carcinoma. It can be concluded that the diagnostic value of the prostate-specific antigen level in serum for the discrimination between benign prostatic hyperplasia and prostate carcinoma was associated with almost 100% specificity and 100% sensitivity. According to the bone scintigraphic data, the carcinoma patients were subdivided into a group with, and a group without, bone metastases. Although the prostate-specific antigen values of these two groups showed some overlap, statistically significantly different median values were measured for the two groups. The median values for the bone markers measured in the three different patient groups are shown in table 2. All analytes yielded values above the upper reference limits in the group of prostate carcinoma patients with bone metastases. These values, compared with those from the group of prostate carcinoma patients without bone involvement, were all statistically significantly raised, except for the urinary calcium excretion. In the two patient groups with benign prostatic hyperplasia and PC− all markers were within the normal reference interval except for the urinary calcium excretion results.

Table 3 shows the *Spearman* rank correlation coefficients of the bone markers in patients with prostatic hyperplasia and prostatic cancer with or without bone metastases. It appeared that the bone formation markers and the bone resorption markers were positively significantly correlated. The urinary calcium excretion results did not correlate at all with the other quantities. In order

to compare the diagnostic value of the different bone markers for the discrimination between prostatic carcinoma patients with and without bone metastases, the individual receiver operating-characteristic curves for each analyte are shown in figure 1. The areas under the curves can be considered as a measure of the diagnostic value of the particular bone marker. These diagnostic values are shown in table 4. Based on the receiver operating-characteristic analysis, cutoff levels for the diagnosis of bone metastasis were also established. These cutoff levels were associated with the maximal diagnostic efficiency (sum of sensitivity and specificity) and are also shown in table 4.

## Discussion

In the present study, seven biochemical markers of bone metabolism were studied in a benign prostatic hyperplasia control group and in prostatic cancer patients with or without scintigraphic evidence of bone metastases. A biochemical marker with the ability to discriminate between patients with and without meta-

**Tab. 1** Characteristics of the patient group with prostate disease. Age and prostate-specific antigen values are given in medians and ranges. Prostate-specific antigen was measured at the time of diagnosis and expressed in µg/l.

| | Prostate cancer with metastasis (PC+) | Prostate cancer without metastasis (PC−) | Benign prostatic hyperplasia |
|---|---|---|---|
| Patients | 19 | 24 | 34 |
| Age | 70 (57−86) | 70 (54−79) | 62 (51−75) |
| Prostate-specific antigen | 710 (17−14000) | 28* (7−424) | 1.6** (0.4−9.6) |
| Bone scintigraphy | positive | negative | − |

* PC− versus PC+: $p < 0.001$ ** Benign prostatic hyperplasia versus PC−: $p < 0.001$

**Tab. 2** Determination of markers of bone turnover in patients with prostate disease. Values are given in medians and ranges.

| | Prostate cancer with metastasis (PC+) | Prostate cancer without metastasis (PC−) | Benign prostatic hyperplasia |
|---|---|---|---|
| *Bone formation* | | | |
| Alkaline phosphatase (U/l) | 210* (76−3863) n = 19 | 79 (46−159) n = 24 | 74 (47−109) n = 34 |
| Bone alkaline phosphatase (U/l) | 80* (17−1884) n = 19 | 23 (10−42) n = 24 | 18 (9−44) n = 33 |
| Carboxyterminal propeptide (µg/l) | 200* (101−500) n = 19 | 119 (91−233) n = 24 | 113 (60−191) n = 33 |
| *Bone resorption* | | | |
| Deoxypyridinoline excretion (µmol/mol creatinine) | 9.2* (4.1−42.6) n = 19 | 4.1 (2.6−7.3) n = 24 | 4.0 (2.3−10.9) n = 32 |
| Aminoterminal telopeptide excretion (µmol/mol creatinine) | 220* (37−1925) n = 18 | 39 (8−138) n = 24 | 31.6 (9−96) n = 22 |
| Carboxyterminal telopeptide (µg/l) | 10.8* (4.6−25.7) n = 19 | 3.3 (1.8−6.4) n = 24 | 2.9 (1.6−6.5) n = 33 |
| Calcium excretion (µmol/mol creatinine) | 206 (34−1363) n = 18 | 392 (47−1042) n = 24 | 272 (103−1145) n = 32 |

* PC+ versus PC− or benign prostatic hyperplasia: $p < 0.001$

**Tab. 3** *Spearman* rank correlation coefficients for the correlation between bone resorption and bone formation markers in patients with prostate disease. All correlation coefficients differed significantly from zero ($p < 0.001$).

| Bone formation markers | Bone resorption markers | | |
|---|---|---|---|
| | Deoxypyridinoline excretion | Aminoterminal telopeptide excretion | Carboxyterminal telopeptide |
| Alkaline phosphatase | 0.612 | 0.627 | 0.624 |
| Bone alkaline phosphatase | 0.423 | 0.647 | 0.496 |
| Carboxyterminal propeptide | 0.654 | 0.779 | 0.625 |

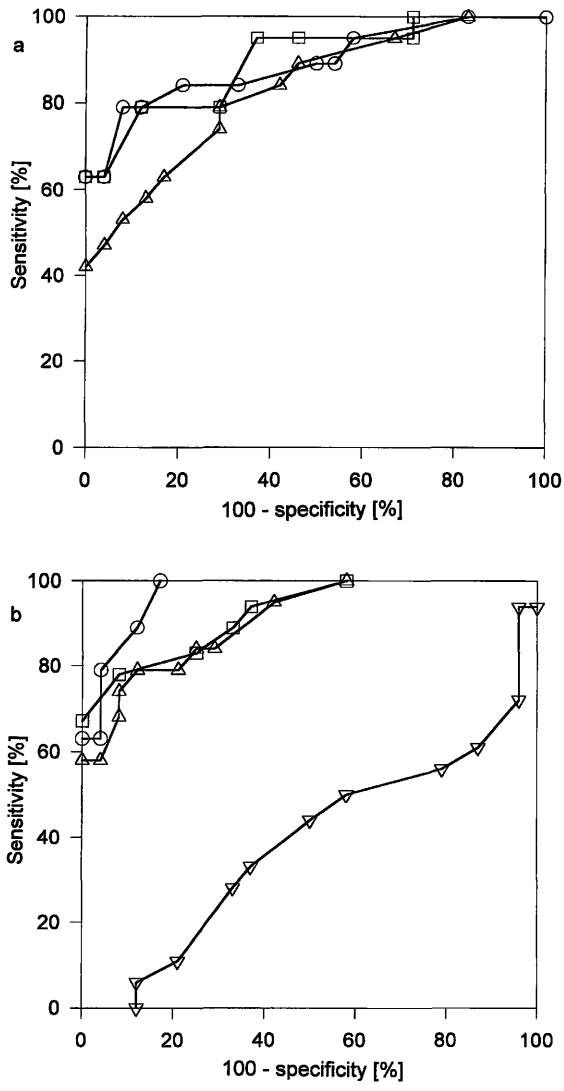


**Fig. 1** Receiver-operating characteristic analysis of bone formation markers (fig. 1a) and bone resorption markers (fig. 1b) for the detection of bone metastases in a group of prostate carcinoma patients with and without scintigraphic evidence for bone metastases.

Fig. 1a: alkaline phosphatase □; bone alkaline phosphatase ○; carboxyterminal propeptide △.

Fig. 1b: Calcium excretion in urine ▽; urinary aminoterminal telopeptide excretion △; urinary deoxypyridinoline excretion □; carboxyterminal telopeptide ○.

static bone involvement would be important for early diagnosis and treatment of advanced cancer of the prostate. The results show that the bone metastatic process in prostate cancer causes a significant rise in biochemical bone formation markers as well as bone resorption markers (tab. 2) and that these markers are mutually correlated (tab. 3). This is in line with the observations that bone involvement in prostatic cancer is mostly caused by increased numbers of active osteoblasts (11) and that the osteoclastic response may be due to increased parathyroid hormone secretion as a consequence of the higher calcium demand in the osteoblastic metastasis (12, 13).

Although not a marker of the primary osteoblastic process, the collagen resorption marker carboxyterminal cross-linked telopeptide of type I collagen shows the highest diagnostic value (tab. 4). A specificity of 88% together with a sensitivity of 89% make this analyte slightly more suitable for the diagnosis of bone metastases, compared for instance with the routinely employed determination of total alkaline phosphatase. Recently, a high specificity of carboxyterminal cross-linked telopeptide of type I collagen for the diagnosis of bone metastases was also reported in a group of prostate cancer patients (9) and in a group of miscellaneous cancer patients (7) but this was associated with a very low sensitivity. This could be caused by the fact that in these studies no receiver operating-characteristic analysis to determine the optimal cutoff level was performed. In a group of heterogeneous cancer patients with bone metastases it has recently been reported that the urinary excretion of collagen deoxypyridinoline cross-links is very promising for the detection or follow up of metastatic bone involvement (3−5). In these studies the carboxyterminal cross-linked telopeptide of type I collagen resorption marker was not taken into account and patients with metastatic prostate carcinoma were not included in two of the studies (3, 4) and only in a very small number (7 patients) in the third study (5). The present results also

**Tab. 4** Diagnostic value, cutoff level, sensitivity and specificity of biochemical bone resorption and formation markers for the diagnosis of bone metastasis in carcinoma of the prostate.

| | Cutoff level | Diagnostic value* | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|
| *Bone formation* | | | | |
| Alkaline phosphatase | 100 (U/l) | 0.90 | 79 | 88 |
| Bone alkaline phosphatase | 30 (U/l) | 0.90 | 79 | 88 |
| Carboxyterminal propeptide | 150 (µg/l) | 0.83 | 74 | 79 |
| *Bone resorption* | | | | |
| Deoxypyridinoline excretion | 7.0 (µmol/mol creatinine) | 0.93 | 78 | 92 |
| Aminoterminal telopeptide excretion | 78 (µmol/mol creatinine) | 0.91 | 79 | 79 |
| Carboxyterminal telopeptide | 4.6 (µg/l) | 0.97 | 89 | 88 |
| Calcium excretion | 200 (µmol/mol creatinine) | 0.39 | 44 | 50 |

* area under the receiver operating-characteristic curve

confirm the good diagnostic value of deoxypyridinoline cross-links for the detection of bone metastases, and it can be concluded that it shows even a better specificity compared with carboxyterminal cross-linked telopeptide of type I collagen or aminoterminal telopeptide of type I collagen. In a recent report a receiver operating-characteristic analysis was performed for the efficacy of urinary pyridinium cross-links for the detection of neoplastic bone disease (6). It was found that the cross-link excretion had a low specificity (ca. 40%), reducing its diagnostic value. However, this result is not comparable with our data on diagnostic values, probably because there were 98 patients with different kinds of bone neoplasia and with heterogeneous primary diseases studied (6).

In one of these studies on heterogeneous cancer patients (3) it was also reported that the measurement of aminoterminal telopeptide of type I collagen was the most suitable assay for predicting the presence of bone metastases although the sensitivity was only about 40%. In the present study we found a much higher sensitivity for the aminoterminal telopeptide of type I collagen assay resulting in a good diagnostic value for the presence of bone metastases, comparable with the results of urinary cross-link excretion tests.

We conclude that the three bone markers carboxyterminal cross-linked telopeptide of type I collagen, deoxypyridinoline cross-links and aminoterminal telopeptide of type I collagen are suitable for the assessment of bone metastases and have a comparable diagnostic value for the assessment of advanced bone resorption. It is clear from our results that the determination of urinary calcium excretion has no added value at all. Bone formation was evaluated by measuring the alkaline phosphatase enzyme from the osteoblast and the carboxy terminal propeptide from type I procollagen. In the present group of patients, alkaline phosphatase and the bone isoenzyme (bone alkaline phosphatase) perform equally well with respect to the diagnosis of advanced prostate carcinoma. Surprisingly, carboxyterminal propeptide of type I procollagen was neither more sensitive nor specific than alkaline phosphatase and bone alkaline phosphatase. This is in line with the results of *Kylmälä* et al. (8), who concluded that the synthesis of a large protein such as type I collagen was possibly disturbed or retarded by cancer cells in advanced malignant disease. It is known that prostate-specific antigen in serum has a high diagnostic value in the early diagnosis of prostate cancer and in the present study it was almost completely possible to differentiate between patients with or without carcinoma of the prostate on the basis of prostate-specific antigen measurements, although the diagnostic value of prostate-specific antigen for detecting bone metastases was limited to 0.87 (receiver operating-characteristic curve not shown). This is also in accordance with the study of *Kylmälä* et al. (8) in which carboxyterminal cross-linked telopeptide of type I collagen surpassed prostate-specific antigen as a prognostic indicator for bone metastases.

In the present study patients were classified in the PC− group because of normal results obtained from bone scintigraphy. Because of this, it is hardly surprising that no pathological median values for the bone quantities could be detected in comparison with the benign prostatic hyperplasia control group. However, in the PC− group, the numbers of elevated values of carboxyterminal propeptide of type I procollagen (but also of aminoterminal telopeptide of type I collagen) were higher in comparison with the benign prostatic hyperplasia group, although these differences did not reach a statistically significant level. Further longitudinal study is needed in order to prove if these increases in carboxyterminal propeptide of type I procollagen or aminoterminal telopeptide of type I collagen reflect early bone involvement before the metastasis is visible in routine bone scintigraphy or before the other biochemical bone markers are raised.

In conclusion, biochemical bone markers are significantly elevated in patients with prostatic bone metastases and show a promising diagnostic value for the detection of osteoblastic or osteoclastic metastatic processes.

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Corresponding author: Dr. L. W. J. J. M. Westerhuis, Department of Clinical Chemistry, Ziekenhuis De Wever & Gregorius, P. O. Box 4446, NL-6401 CX Heerlen, The Netherlands